UNITED STATES DEPARTMENT OF COMMERCE
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Washington, D.C. 20231

08/886,044

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SERIAL NUMBER	FILING DATE	FIRST NAMED APPL.	ATTORNEY DOCKET NO.
08/886,044	06/30/97	BHATTACHARJEE A	710007/137

HM12/0601

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ART UNIT	PAPER NUMBER
1641	40

DATE MAILED: 06/01/00

Below is a communication from the EXAMINER in charge of this application

COMMISSIONER OF PATENTS AND TRADEMARKS

ADVISORY ACTION

☐ THE PERIOD FOR RESPONSE:

a) ☐ is extended to run ______ or continues to run ______ from the date of the final rejection

b) ☐ expires three months from the date of the final rejection or as of the mailing date of this Advisory Action, whichever is later. In no event however, will the statutory period for the response expire later than six months from the date of the final rejection.

Any extension of time must be obtained by filing a petition under 37 CFR 1.136(a), the proposed response and the appropriate fee. The date on which the response, the petition, and the fee have been filed is the date of the response and also the date for the purposes of determining the period of extension and the corresponding amount of the fee. Any extension fee pursuant to 37 CFR 1.17 will be calculated from the date of the originally set shortened statutory period for response or as set forth in b) above.

☒ Appellant's Brief is due in accordance with 37 CFR 1.192(a).

☒ Applicant's response to the final rejection, filed 04/18/2000 has been considered with the following effect, but it is not deemed to place the application in condition for allowance:

1. ☐ The proposed amendments to the claim and /or specification will not be entered and the final rejection stands because:

a. ☐ There is no convincing showing under 37 CFR 1.116(b) why the proposed amendment is necessary and was not earlier presented.

b. ☐ They raise new issues that would require further consideration and/or search. (See Note).

c. ☐ They raise the issue of new matter. (See Note).

d. ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal.

e. ☐ They present additional claims without cancelling a corresponding number of finally rejected claims.

NOTE: ______

2. ☐ Newly proposed or amended claims ______ would be allowed if submitted in a separately filed amendment cancelling the non-allowable claims.

3. ☒ A Notice of ~~Upon the filing an~~ appeal having been filed, the proposed ~~amendment~~ response ☒ will be entered ☐ ~~will not be entered~~ and the status of the claims will be as follows:

Claims allowed: None
Claims objected to: None
Claims rejected: 1-3, 5-8 and 15-17.

However;

☐ Applicant's response has overcome the following rejection(s): ______

4. ☐ The affidavit, exhibit or request for reconsideration has been considered but does not overcome the rejection because See Attached Sheets.

5. ☐ The affidavit or exhibit will not be considered because applicant has not shown good and sufficent reasons why it was not earlier presented.

☐ The proposed drawing correction ☐ has ☐ has not been approved by the examiner.

☒ Other Interview Summary (paper no. 39).

ATTACHMENT TO ADVISORY ACTION

Applicants' Request for Reconsideration

1) Acknowledgment is made of the Applicants' request for reconsideration filed 04/18/2000 (paper no. 38) in response to the Final Rejection mailed 08/19/2000 (paper no. 30).

Status of Claims

2) Claims 19 and 20 were canceled via paper no. 33 filed 01/19/2000.

Claims 1-3, 5-8 and 15-17 are under examination.

Rejections Moot

3) The rejections of claims 19 and/or 20 made in paragraphs 8-12 of the Office Action mailed 08/19/2000 (paper no. 30) under various statutes are moot in light of Applicants' cancellation of the claims.

Rejection Maintained

4) The rejection of claims 1-3, 5-8 and 15-17 made under 35 U.S.C. § 103(a) as being unpatentable over Zollinger *et al.* (US 4,707,543) in view of Ziegler *et al.* (*New Eng. J. Med.* 307: 1225-1230, 1982) or Myers *et al.* (US 4,912,094) and Munford *et al.* (US 4,929,604) is maintained.

Applicants' Arguments & the Office's Response

5) Applicants cite and discuss multiple references, which were not applied in the rejection(s) made by the Office. However, in order to effectively address Applicants' concerns and to clarify the issues further, the Office has provided the rebuttal herebelow by citing or discussing extra references as needed. It should be noted that none of these extra references are applied in the rejection, but are cited solely to rebut the evidence supplied by Applicants and to reflect what is already known in the art.

Instant Invention

Instant claims are drawn to a vaccine effective in actively immunizing a subject against infection by heterologous Gram-negative bacteria, or the lipopolysaccharide (LPS) "endotoxin-mediated pathology" by production of an "antibody", comprising a non-covalent complex of purified detoxified *E. coli* J5 LPS and a purified outer membrane protein (OMP) derived from

Neisseria meningitidis and a method of using the vaccine for active immunization of a subject.

It is important to note that, in their response filed 04/10/2000 (paper no. 38) and in the Declaration by Dr. Alan Cross filed 04/27/99 under 37 C.F.R § 1.132, Applicants state that the instant vaccine "prevent neither systemic infection nor initiation of sepsis", but "significantly reduce the likelihood of a lethal outcome following infections with both heterologous strains of bacteria". These effects are not considered 'unexpected' or 'surprising', since LPS-containing vaccines of the prior art have accomplished similar effects. Applicants further specify that antibodies "produced in response to vaccination with applicants' vaccine do not appear to promote killing of bacteria, either directly or indirectly" (see page 13 of the response and page 2 of the Declaration). Further, Applicants assert that the antibodies elicited by Applicants' vaccine bind to *Klebsiella* and *Pseudomonas* (see page 10 of the Applicants' response filed 04/10/2000). Such cross-reactivity or binding of J5 antibodies to heterologous Gram negative bacteria is not considered 'unexpected' or 'surprising', since the J5-induced antibodies of the prior art have shown such binding. See below under paragraph 11 for prior art teachings.

6) Applicants allege that the Office has misapplied the primary reference of Zollinger *et al.* (US 4,707,543). Applicants argue that the Office has applied Zollinger *et al.* "in a selective manner", picking OMP from *N. meningitidis* and detoxified LPS from *E. coli*. Applicants further contend that this "mixing and matching is not suggested by Zollinger" (see pages 3 and 4 of the Applicants' response filed 04/18/2000). Applicants further allege that the Office's position that Zollinger *et al.* teach a combination of OMP from *N. meningitidis* and LPS from *E. coli* is a "hindsight reconstruction" of the art and that such a combination "falls outside the ambit of Zollinger's teachings" and "is contrary to Zollinger's express purpose" (see second paragraph on page 5 of the Applicants' response filed 04/18/2000). Applicants further state that the "use of outer membrane protein and polysaccharide from the ***same species*** is consistent with another Zollinger teaching, that the vaccine is directed against the same species from which the OMP is obtained" (see the first paragraph on page 5 of the Applicants' response filed 04/18/2000) [Emphasis in original].

A thorough review of the patent of Zollinger *et al.* ('543) reveals that, in Example 3, Zollinger *et al.* teach a non-covalent complex of OMP and detoxified polysaccharide obtained

from the same species. However, Zollinger *et al.* also explicitly teach a vaccine composition comprising OMP from one Gram negative bacterial pathogen such as serogroup B *N. meningitidis,* and polysaccharide from another Gram negative bacterial pathogen such as serogroup C *N. meningitidis*, or serogroup C, Y, Y and W-135 *N. meningitidis*. See Examples 1 and 2, and Table H. It was well known in the art that serogroup B *N. meningitidis* is serologically, biologically and/or immunogenically a heterologous or distinct Gram negative bacterial pathogen from serogroup C, Y, Y or W-135 *N. meningitidis*. In Example 2, Zollinger *et al.* teach a noncovalent complex of two serogroup B meningococcal OMPs and a tetravalent mixture of polysaccharides from four serologically distinct Gram negative bacterial pathogens, i.e., groups A, C, Y and W-135 *N. meningitidis.* Zollinger *et al.* state that such a vaccine "might protect against meningococcal disease of **all 5** pathogenic serogroups (A, B, C, Y and W-135)" (see column 7, lines 59-68) [Emphasis added].

In this context, it is important to note that Zollinger *et al.* define the term "polysaccharide" as including "lipopolysaccharides and capsular polysaccharides" (see column 2, lines 27-29). The vaccine is used safely in a method of active immunization of humans, i.e., military recruits (see Example 2 in column 8). Furthermore, Zollinger *et al.* expressly teach that the process of the invention is "generally applicable to the preparation of **detoxified** polysaccharide-protein complexes derived from gram-negative bacteria" and that *"Neisseria meningitidis* group B" and "*Escherichia coli*" are "preferred" (see column 4, lines 23-29). Most importantly, under the section 'Utility' in column 12, lines 17-25, Zollinger *et al.* disclose:

> The detoxified polysaccharide-outer membrane protein complexes prepared according to applicants' novel process of this invention **induce immune response to bacterial infections. More specifically,** evidence indicates that these **complexes have activity against bacterial infections caused by gram-negative** bacteria including ***Neisseria meningitidis group B***, *Haemophilus influenzae type b, Neisseria gonorrhoeae,* ***Escherichia coli*** and *Pseudomonas aeruginosa.* [Emphasis added].

Further, the heterologous Gram negative bacterial source of the two critical components of Zollinger's vaccine is apparent from the claims. Zollinger's claims 5, 7 and 8 indicate that the detoxified lipopolysaccharide-OMP complex may be derived from one or more strains of gram-negative bacteria selected from the group consisting of group B *N. meningitidis* and *E. coli*, and

claims 23, 24 and 25 teach that the purified OMP is obtained from group B *N. meningitidis*. Therefore, the purpose of Zollinger' OMP-polysaccharide or detoxified polysaccharide is not only to prepare a vaccine for active immunization against the same bacteria, but also to provide protection against "infection" (including LPS "endotoxin-mediated pathology) caused by heterologous Gram negative bacteria.

Thus, the Office has not "misapplied" Zollinger *et al.* as the primary reference and has not used hindsight reconstruction. The Office has NOT applied Zollinger *et al.* in a "selective manner" as Applicants allege. Instead, Zollinger *et al.* themselves teach explicitly what Applicants view as "mixing and matching" of OMP, i.e., detoxified polysaccharide vaccine components from heterologous Gram negative bacterial pathogens including *Escherichia coli* and *Neisseria meningitidis* group B. In fact, the detoxified group B meningococcal lipopolysaccharide component, present in Zollinger's vaccine, was shown in the art to carry a core region that is "immunologically similar" to *E. coli* J5 LPS. For instance, Davis *et al.* (Davis, Ziegler and Arnold) (*J. Exp. Med.* 147: 1007-1017, 1978, abstract, already of record) taught the immunological similarity between the meningococcal LPS core and enteric LPS. Davis *et al.* taught that purified J5 LPS antibodies neutralized meningococcal endotoxin and suggested that these J5 antibodies could interrupt the devastating course of meningococcal endotoxemia in man (see abstract). Thus, J5 LPS core is a functional (i.e., immunological) equivalent of Zollinger's meningococcal LPS core. This is evidence that the patent of Zollinger *et al.* is properly applied by the Office in the rejection made. Therefore, the Office maintains that a *N. meningitidis* OMP- *E. coli* detoxified LPS non-covalent vaccine combination falls well within the ambit of Zollinger's teachings and is consistent with Zollinger's express purpose of providing protection to multiple Gram negative bacterial pathogens, including meningococci and *E. coli*, all of which are known causative agents of "LPS endotoxin-mediated pathology" and Gram negative bacterial "infections".

7) Applicants contend that the ***"purpose*** of the polysaccharide in Zollinger, whether capsular polysaccharide or lipopolysaccharide, is to solubilize the outer membrane proteins" (see 4 of the Applicants' response filed 04/18/2000) [Emphasis in original]. Applicants further state (see the first paragraph on page 5 of the Applicants' response filed 04/18/2000):

> If the polysaccharide is to fulfill any purpose in addition to the solubilization function taught by Zollinger, the clear indication in the reference is that it serve to strengthen the antigenic response to the OMP. This would best be achieved by using polysaccharide from the same species as the OMP.

In light of what is known in the art, the detoxified polysaccharide used by Zollinger *et al.* is less likely to enhance or strengthen the antigenic response to the OMP. Instead, the group B meningococcal OMP has been shown to serve as a potent adjuvant and an effective protein carrier to poor immunogens such as polysaccharides, on covalent or non-covalent formulation. See Liu *et al. PNAS* 89: 4633-4637, 1992; Moreno *et al. Infect. Immun.* 47: 527-533, 1985; and Hawe *et al. In*: *Proceedings of Modern Approaches to New Vaccines*, Cold Spring Harbor laboratories, Cold Spring Harbor, N.Y., pp. 76, 1992, all which teach group B meningococcal OMP to be a bacterially derived immunostimulant or adjuvant or immune-enhancing protein carrier. Thus, it is reasonable to conclude that Zollinger's meningococcal OMP inherently acts as an adjuvant and enhances the host immune response to the detoxified polysaccharide present in the OMP-polysaccharide complex. In fact, Zollinger *et al.* themselves teach that the "antibody response" to the polysaccharide in mice actively immunized with the heterologous OMP-polysaccharide complex "was enhanced" in the "protein-containing vaccine" (see column 7, lines 40-42).

Thus, the polysaccharide component in Zollinger's vaccine serves not only as a solubilizing agent, but also as an effective immunogen by its presentation to the immune system along with the bacterially derived immunostimulant, group B meningococcal OMP.

8) Applicants further allege that even if Zollinger *et al.* taught the use of detoxified LPS from *E. coli* non-covalently complexed with OMP of group B *Neisseria meningitidis* as a vaccine against infection, one skilled in the art would not have been motivated to substitute J5 LPS of Ziegler in place of Zollinger's generic *E. coli* LPS, for the expected benefit of using an immunogen that elicits protective response against multiple pathogenic bacterial species. Applicants contend that there would have been no reasonable expectation of success in using such a composition in active or passive immunization against Gram negative bacterial sepsis. Applicants state that the teachings of Munford and Myers (the two secondary references applied by the Office in the rejection) that the R core region of LPS is similar in most gram negative bacteria "were debunked in the art when the present application was filed" (see page 3 of the

Applicants' response filed 04/18/2000).

It is widely known in the art of Gram negative bacteria and LPS Immunology that the core region of LPS is similar in Gram negative bacteria. This fact has been demonstrated structurally, functionally, biologically and/or immunologically by several skilled practitioners in the art including Ziegler *et al.*, Munford *et al.*, and Myers *et al.* In fact, the detoxified group B meningococcal lipopolysaccharide component, present in Zollinger's vaccine, was shown in the art to carry a core region that is "immunologically similar" to *E. coli* J5 LPS. See the teaching of Davis *et al.* (*J. Exp. Med.* 147: 1007-1017, 1978, already of record) explained above in paragraph 7.

First, Applicants themselves agree on page 10, first paragraph of their response that Myers *et al.* teach the highly conserved nature of the core region of LPS by stating that "[t]his fact has long been known". Applicants cite an unspecified citation of Lugowski, with a post-filing publication date of 1996, as teaching microheterogeneity in the LPS core epitopes. Applicants point to "Figure 11" of the "Lugowski" publication and to "studies by Lugowski *et al.* (1996)". Applicants agree that Lugowski's LPS core antiserum did show cross reaction with "other cores from *E. coli*" including the core prototype of J5. Assuming that Applicants are referring to the data depicted in Figure 3 of the publication of Lugowski *et al. FEMS Immunology and Medical Microbiology* 16: 21-30, 1996, the Figure convincingly demonstrates that, despite the stated structural microheterogeneity, an antiserum raised to *E. coli* R3 (J5) conjugate, in the absence of a strong adjuvant such as group B meningococcal OMP, cross-reacted well with the LPS of heterologous Gram negative bacteria including that of *Sh. flexneri*, *Citrobacter*, R2 core prototype of *E. coli*, *E. coli* O111 serotype, and to some extent, also with the LPS of *Klebsiella pneumoniae*, *S. typhimurium* and Ra prototype of *E. coli*. It has also been shown in the past that despite structural microheterogeneity, cross-reactive antibodies can be produced to the conserved region of the core polysaccharide. For instance, Lugowski *et al.* (*J. Immunol. Methods* 95: 187-194, 1986) teach that *E. coli* O14 core oligosaccharide shows microheterogeneity by differing from the *Shigella sonnei* phase II core oligosaccharide only in one structural element, the terminal beta-hexosyl residue, yet it does cross-react with the *Shigella sonnei* phase II core oligosaccharide-induced antiserum (see page 194 and Figure 4). Lugowski *et al.* (1986) thus

provided the serological confirmation of the highly conserved nature of the core LPS among Gram negative bacteria.

The applied prior art, Ziegler *et al.* (*New Eng. J. Med.* 307: 1225-1230, 1982) teach that the "LPS of *E. coli* J5 lacks oligosaccharide side chains and that its core, which is exposed, is nearly identical to that of most other gram-negative bacteria" (see abstract). The J5 LPS-induced antibodies "conferred protection against Shwartzman reactions caused by purified endotoxins from bacterial species as widely varied as *E. coli*, *Salmonella typhimurium*, and the meningococcus" (see page 1226), i.e., the heterologous gram negative bacteria recited or encompassed in the base claims.

The applied prior art, Myers *et al.* (US 4,912,094) teach that "the core region is highly conserved among LPSs obtained from different genera of *Enterobacteriaceae*" and that "immunity against the core region is ..... protective against a wide variety of Gram negative bacterial challenges" and "was demonstrated by the work of Ziegler *et al.*" (see column 2, lines 9-13). Most importantly, Myers *et al.* explicitly teach that "LPS prepared .... from a strain that has a partially-complete (and therefore antigenically cross-reactive) core-region (e.g. *E. coli* J5)" can be used as a vaccine against gram-negative infections (column 10, lines 4-9) [Emphasis added]. Myers *et al.* teach how to detoxify Gram negative bacterial LPS to reduce its toxicity. Additionally, Myers *et al.* expressly teach that such a modified LPS, which is rendered less toxic, can be used "as a vaccine against gram negative infections", with or without "other bacterially-derived immunostimulants" (see columns 10 and 1, first full paragraphs). It is noteworthy that, because of its antigenic cross-reactivity, Myers *et al.* specifically picked detoxified *E. coli* J5 as a vaccine component for administration against Gram negative infections along with a bacterially-derived immunostimulant. Myers *et al.* thus specifically direct one skilled in the art to select *E. coli* J5 LPS (detoxified) as a vaccine component. Note that Applicants' vaccine composition comprises detoxified *E. coli* J5 LPS combined with a known "bacterially-derived immunostimulant", such as group B meningococcal OMP, for active immunization against Gram negative infections. It should be noted that in the rejection made by the Office, the references of Ziegler *et al.* and Myers *et al.* were used as alternative references, and Ziegler's J5 LPS and Myer's J5 LPS were used as alternative LPSs for substitution in Zollinger's composition (see

paragraph 8 of the Office Action mailed 09/14/98).

The applied prior art, Munford *et al.* (US 4,929,604), teach that "the structure of the lipid A moiety is highly conserved" in the LPS of many pathogenic bacteria including *Salmonella, Escherichia, Haemophilus* and *Neisseria*, and that LPSs may be used as vaccines to prevent gram negative bacterial sepsis by producing antibodies to R-core regions (see the abstract and column 2, lines 41-50). Munford *et al.* teach that the structure of the R core region of LPS "is similar in most gram negative bacteria" (see column 1, lines 34-36).

Thus, within the cited art, the Office has established the motivation for J5 substitution in Zollinger's vaccine to obtain the vaccine composition of the instant invention, with a reasonable expectation of success. Ziegler *et al.*, Myers *et al.*, and Munford *et al.* individually provide the express motivation for one skilled in the art to use Ziegler's or Myer's J5 LPS in Zollinger's vaccine composition to produce the instant invention. The Office has clearly met the burden of making a *prima facie* case of obviousness.

The following are additional references that document teachings similar to that of the cited references, with regard to the conserved nature of the LPS core.

- Cohen *et al.* (*Lancet* 1: 8-11, 1987) teach that the "core glycolipid of endotoxin has essentially the same structure in all gram-negative bacteria" (see page 8, right column).

- Marks *et al.* (i.e., Marks, Ziegler and Douglas) (*J. Clin. Invest.* 69: 742-749, 1982) teach that "all gram-negative bacteria have similar LPS core structures" (see abstract) and that "core regions of LPS from a variety of enteric bacilli are structurally related" (see page 742, right column). Marks *et al.* further teach that "lipid A from LPS of several enteric and nonenteric gram-negative bacteria including *H. influenzae* are antigenically similar" (see page 742, right column).

- Tyler *et al.* (Immunity targeting common core antigens of gram-negative bacteria. *J. Vet. Internal Med.* 4: 17-25, 1990) teach:

> **In contrast to the heterogeneity of somatic antigens, underlying gram-negative core antigens possess marked chemical, structural, and immun logic homology acr ss species, genera, and group, making these**

> **antigens attractive candidates as cross-protective immunogens.** Lipid A, N-acetylglucosamine, 2-keto-3-deoxyoctonate (KDO), heptose, and glucose residues **are highly uniform, even across a broad spectrum of distantly related gram-negative bacteria.**[33,34] (page 18, right column). [Emphasis added].

♦ Tyler *et al.* (*Am. J. Vet. Res.* 49: 1950-1954, 1988) teach, with regard to *E. coli* J5:

> Core antigens have marked structural and immunologic uniformity,[1,4] creating the potential for cross-reactive immunoprophylaxis to syndromes caused by opportunistic gram-negative bacteria. One such syndrome is bovine gram-negative bacterial mastitis, commonly called coliform mastitis[5-10] (see abstract).
> ....gram-negative cell wall core-antigen components (lipid A, KDO, hexose, heptose, phosphate, and ethanolamine) have structural and antigenic homogeneity across bacterial variety, species, genera, and groups.[4,25,26] (see page 1953).
> Three requirements must be met before immunoprophylaxis to gram-negative mastitis can be used. First, a common antigenic structure must be shared by potential pathogens. Second, this antigen must function as an immunogen, inducing a humoral or cell-mediated response. Third, this response must prevent infection or reduce severity of clinical disease. .... Thus, the core antigen from an R-mutant gram-negative bacteria at least partially satisfies the requirements of shared antigen structure, immunogenicity, and immunoprophylaxis (see page 1953).

Thus, in 1988, Tyler *et al.* provided the motivation for using *E. coli* J5 LPS in a vaccine composition for mastitis (i.e., endotoxin-mediated pathology).

♦ McCabe *et al.* (*Progr. Clin. Biol. Res.* 47: 107-117, 1980, already of record) teach:

> ...there was marked similarity of the core portion of the LPS of all Enterobacteriaceae. Although slight variations in sugar composition of the core portion may be observed among LPS from various species, the inner portion comprised of lipid A, ketodeoxyoctonate (2-keto-3-deoxyoctulosonic acid,[KDO]) and heptose appears identical in LPS from all Enterobacteriaceae (Luderitz et al., 1966). Thus, the LPS isolated from Re, Rd_1, and Rd_2 mutants is common to the LPS of all Enterobacteriaceae. Demonstration that components of LPS were shared by all Gram-negative bacilli suggested that, if these were exposed on the bacterial surface, all Enterobacteriaceae might possess shared antigenic determinants (see page 109).

♦ Teng *et al.* (*PNAS* 82: 1790-1794, 1985, see abstract) teach that the J5 mutant of *E. coli* is deficient in O-antigenic side chains and that this deficiency exposes the core oligosaccharide, which is common to LPS of all Gram-negative bacteria. Nelson *et al.* also teach that lipid A represents the most conservative and least variable structural element of LPS (see abstract).

9) Applicants contend that "***all*** previous attempts to immunize or otherwise protect individuals against LPS endotoxin-mediated pathology had been unsuccessful" [Emphasis in original]. Applicants produce a post-filing (1997) review article by Greisman *et al.* as substantiating their statement. Applicants acknowledge, however, that (see page 6 of the

Applicants' response filed 04/18/2000):

> As to each and every study implicating broad-spectrum protection by rough-mutant antisera, Dr. Greisman concluded that **defects, in design or methodology,** had engendered **inconsistent results** and that antisera to the J5 chemotype "do not appear capable of providing broad-spectrum protection". [Emphasis added].

As Greisman *et al.* do, many in the art agree that some of the inconsistent results obtained in the art are due to the defects in the study design and methodology. For instance, Cross (one of the inventors) and Opal (*J. Endotoxin Res.* 1: 57-69, March 1994, already of record) pointed out that the choice of target population and premature termination of studies when there was barely sufficient numbers of patients to provide adequate statistical power to each study, put the entire study result into question. Additionally, the leaky mutation in a J5 isolate (Cross *et al., J. Endotoxin Res.* 1: 57-69, March 1994), the length of growth or the cultural maturity of J5 culture (McCullus *et al. Infect. Immun.* 55: 1042-1046, 1987, abstract; Ziegler *et al., J. Infect. Dis.* 158: 286-290, 1988, see page 288, of record; and Tyler *et al., J. Vet. Intern. Med.* 4: 17-25, 1990, see page 18, last paragraph) have also been recognized as influencing the expression of cross-reactive/cross-protective epitopes in J5, and thus the outcome or conclusion of clinical studies.

Applicants as well as Greisman *et al.* discuss a few negative studies obtained with the anti-lipid A monoclonal antibody, HA-1A, and state that one of skill in the art would not have been motivated to use *E. coli* J5 LPS in Zollinger's composition. However, it should be noted that the instantly claimed vaccine comprises *E. coli* J5 LPS that is detoxified or modified.

A review of the of Greisman's publication (1997) suggests that this review article is incomplete in that it fails to cite and/or discuss a plethora of positive studies, published in the art prior to the filing of the instant application. These studies demonstrate that J5 bacterin, J5 LPS alone, or J5 LPS in combination with a protein or an adjuvant, do induce antibodies that are cross-reactive/cross-protective. Some of the studies not included or discussed in the Greisman review article and undisclosed to the Office by the Applicants were already cited in the last Office Action mailed 08/19/1999 (paper no. 30) solely to rebut Applicants' arguments. Those references and others are provided in this Office Action as a part of further addressing the Applicants' rebuttal.

A) Moore *et al.* (*Transplantation* 44: 249-253, 1987, already made of record) teach

that endotoxin (ET) has a role in the pathogenesis of graft-versus-host disease (GVDH). Moore *et al.* teach the benefits of active and passive immunizations to modulate the severity of GVDH (i.e., an endotoxin mediated pathology) using purified J5 LPS. Moore *et al.* teach active immunization of a mammalian subject against bacterial endotoxin using pure *E. coli* J5 LPS as an immunogen, and passive immunization with an anti-J5 antiserum (see abstract and page 250). Active immunization with purified J5 LPS alone, without complexing or conjugation with any protein, produced a significant increase in J5 antibody level (P=0.0001) (see page 251). Notably, active immunization both with the purified J5 LPS and J5 cells protected against weight loss from GVHD ($P<0.05$) (see page 252).

B) Cryz *et al.* (*Eur. J. Clin. Microbiol.* 4: 180-185, 1985) demonstrated, by active immunization, that an O-polysaccharide-deficient lipopolysaccharide derived from *E. coli* J5 LPS (administered in the absence of a strong adjuvant such as group B meningococcal OMP) is immunogenic in mice. This *E. coli* J5 LPS, used in the immunogenicity and cross-protection studies, was obtained from Dr. E. Ziegler (see page 181). Mice immunized with J5 LPS alone showed 70% protection against challenge with a heterologous Gram negative bacterium, such as *Pseudomonas aeruginosa* E576. The death rate compared to alum-injected (as opposed to saline-injected) control group was decreased by more than 50% and this protection could be attributed to the production of specific IgG which recognized LPS from *Pseudomonas aeruginosa* E576 (see abstract; Results; page 182; Table 2 and page 183, last paragraph). This study provided the biological evidence of cross-reactivity and cross-protection, against a heterologous Gram negative pathogen, afforded by an unconjugated or non-complexed purified J5 LPS.

C) Marks *et al.* (i.e., Marks, Ziegler and Douglas) (*J. Clin. Invest.* 69: 742-749, 1982) teach the cross-protective potential of *E. coli* J5 LPS core against *Haemophilus influenzae* type b infection (see abstract). Marks *et al.* teach a method of actively immunizing rabbits with a preparation of purified J5 LPS alone, without conjugating or complexing with any protein. The core-specific antibodies in the resultant antiserum were measured using erythrocytes coated with "alkaline-treated J5 LPS", i.e., detoxified J5 LPS (see page 743 under 'Methods'). This J5 LPS antiserum showed as potent protection in two experiments (69% and 89% survival) against death due to heterologous *Haemophilus influenzae* type b infection as the antiserum to whole *E. coli*

J5 cells (62% and 86% survival) (see abstract and Table VII). Via active immunization with heat-killed *E. coli* J5, adsorption experiments and the protection afforded by antiserum prepared against purified J5 LPS, Marks *et al.* further provided the convincing biological evidence that J5 LPS core is the essential protective (i.e., cross-protective) determinant. These data exclude non-specific stimulation as the mechanism of protection with J5 vaccine and avoid problems in interpretation arising from vaccine contamination with non-LPS immunogens (see abstract and page 745, left column).

It is important to note that, in 1982, Marks *et al.* recognized the potential for toxicity with a purified LPS immunogen, but reminded that LPS-containing vaccines, such as a pertussis and typhoid, have been used in the past with some success. To overcome this problem, Marks *et al.* suggested that "efforts can be directed at reducing attendant toxicity", i.e., detoxification. Marks *et al.* also provided the motivation for one of skill in the art for choosing *E. coli* J5 LPS by teaching that *E. coli* J5 is "a readily available and well-characterized source of core glycolipid" which has "previously been shown to confer cross-protective effects against diverse gram-negative bacteria and endotoxin (9-14)" (see page 747, left column). Note that, of these cited references, 12-14 are of Ziegler *et al.* and reference 11 is of Davis *et al.* (*J. Exp. Med.* 147: 1007-1017, 1978, already of record).

D) Tomita *et al.* (Immunization of dairy cows with an *Escherichia coli* J5 lipopolysaccharide conjugate vaccine against coliform mastitis. *J. Dairy Sci.* 76: Suppl. pp. 159, abstract P36G, 1993) teach the synthesis and immunological response of a *E. coli* J5 detoxified lipopolysaccharide conjugate vaccine. The detoxified J5 LPS is 2500-fold less toxic than the native LPS and is conjugated to a protein. Dairy cows immunized with the conjugate showed an enhanced IgG immune response comparable to that of cows immunized with J5 bacterin (see entire abstract).

E) Tomita GM (Immunization of dairy cows against coliform mastitis. *Dissertation*, The Ohio State University, 1994) teaches a vaccine comprising **detoxified** *E. coli* J5 bound (covalently) to a carrier protein and an *E. coli* J5 bacterin. On active immunization of mammals (cows), both the conjugate vaccine and the *E. coli* J5 bacterin elicit J5 LPS-specific IgM and IgG antibodies. The precipitated serum IgG induced by both vaccines strongly cross-reacted with

whole cell antigens of heterologous Gram negative bacterial pathogens including *Klebsiella pneumoniae*, *Enterobacter* and *Serratia marcescens* (see Figures 15-19 and Figures 71 and 72).

F) Tomita *et al.* (*J. Dairy Sci.* 78: 2745-2752, 1995, already of record) teach the isolation of serum IgG from cows immunized with an *E. coli* J5 detoxified LPS conjugate vaccine and show that it is highly cross-reactive with the LPS of *E. coli* J5, *E. coli* O111:B4, *Serratia marcescens*, *Klebsiella pneumoniae* and *Salmonella typhimurium* (see abstract).

The above-cited references effectively also rebut the Applicants' statement to the Office (see page 6 of Applicants' amendment filed 01/14/99) that "[a]ll of the studies" listed in a review article by Cross *et al.* "used whole, killed bacterial preparations. **None used purified LPS** either alone or formulated with another component" [Emphasis added]. This shows that the cited review article of Cross *et al.* is missing prior art references that taught the use of "purified LPS either alone or formulated with another component" for immunization of a subject. A detoxified J5 LPS vaccine was already shown to contain cross-reactive epitope(s) which induced antibodies that are cross-reactive with multiple heterologous Gram negative bacterial pathogens including *Klebsiella* and *Pseudomonas*, and their LPSs.

G) Dunn *et al.* (*Surgery* 96: 440-446, 1984, already of record) used purified J5 LPS (and J5 cells) as an immunogen in a mammal to raise antibodies that are cross-reactive *in vitro* and cross-protective *in vivo* against sepsis due to heterologous Gram negative bacteria including *Klebsiella* and *Pseudomonas*. Dunn *et al.* "*sought to test the ability of equine antibody directed against core endotoxin, a portion of bacterial outer membrane lipopolysaccharide common to many gram-negative microorganisms, to bind to various gram.-negative bacteria in vitro, to promote bacterial phagocytosis by leukocytes, and to protect against lethal gram-negative bacteremia in mice*" (Emphasis in original). Dunn *et al.* further teach that (see abstract):

> *Preimmunization IgG and F(ab')2 possessed no titer as determined by enzyme-linked immunosorbent assay, did not promote in vitro phagocytosis, and did not protect in vivo. Postimmunization IgG and F(ab')2 possessed a significant titer to E. coli J5 whole cell and lipopolysaccharide antigens and provided significant ($p < 0.05$) protection in vivo during lethal intravenous sepsis caused by either E. coli J5, E. coli O111:B4, Klebsiella pneumoniae, or Pseudomonas aeruginosa.*

The postimmunization IgG promoted *in vitro* phagocytosis of *E. coli* J5, *E. coli* O111:B4, *Klebsiella pneumoniae,* or *Pseudomonas aeruginosa.* Dunn *et al.* state (see pages 443 and 444):

> In the present study we demonstrated that immunization with *E. coli* J5 led to the development of immune antibody, which reacted primarily to the immunizing strain of bacteria but also extensively cross-reacted to a variety of serotypically distinct gram-negative microorganisms and types of LPS. Similar cross-reactive enhancement of phagocytosis was noted when immune IgG was compared with preimmunization IgG. .... Pretreatment with either immune IgG or F(ab')2 before induction of sepsis conferred cross protection to four challenge organisms, three that were serotypically distinct from *E. coli* J5.
>
> Our results thus demonstrated that purified IgG directed against *E. coli* J5 whole cell and LPS antigens was cross-reactive in vitro and cross protective in vivo.

H) Jachymek (*Postepy Hig Med Dosw* 49(1): 171-178, 1995) teach:

> Another approach to the treatment and prevention of septicaemia involves stimulation of an immune response against LPS. It was found that **immunization with core structures of endotoxin conjugated with proteins protected animals against infections and endotoxic shock. Anticonjugate sera** are of great interest because they **are directed against common parts of LPS** and therefore **could have cross-reactive and cross-protective potencies towards many Gram-negative rods** [see abstract]. [Emphasis added].

I) Spier *et al.* (*Circ. Shock* 28: 235-248, 1989, abstract) teach protection against clinical endotoxemia in horses by using J5 bacterin-induced hyperimmune plasma containing antibody to an Rc mutant *E. coli* (J5). Spier *et al.* teach (see abstract):

> Horses receiving J5 hyperimmune plasma had a significantly improved clinical appearance 48 hours after plasma administration ($P<0.05$) and a shorter period to recovery than control horses ($P=0.069$).

J) Dale *et al.* (*J. Infect. Dis.* 166: 316-325, 1992, already made of record) demonstrated bactericidal antibodies against a serum-resistant strain of *Neisseria gonorrhoeae* lipopolysaccharide by actively immunizing a human volunteer with *E. coli* J5 mutant (see abstract). There was a ten-fold increase in IgG anti-J5 antibody that peaked at nine months.

K) Wickstrom *et al.* (*Vet. Microbiol.* 13: 259-271, 1987, abstract) teach the role of cross-reactive antibody elicited by *E. coli* J5 mutant in immunity to colisepticemia in calves. Antiserum cross-reactive with different serotypes of *E. coli* was produced in cattle immunized with *E. coli* J5 mutant and was given to hypogammaglobulinemic calves before oral challenge with virulent *E. coli* derived from a septicemic calf. Calves passively immunized with bovine anti-J5 serum had delayed and decreased bacteremia and also lived longer than saline-administered controls (see abstract).

L) Schwartzer *et al.* (*J. Infect. Dis.* 158: 1135-1136, 1988) teach immunization of healthy adult male volunteers with a safe, heat-killed *E. coli* J5 vaccine. In 50% of the vaccinees,

a fourfold or greater increase in anti-J5 LPS antibody response was mounted (see page 1135).

M) Cohen *et al.* (*Lancet* 1: 8-11, 1987) teach that a "rough-mutant strain, *Escherichia coli* J5, has only core determinants in its endotoxin, and antibodies to *E. coli* J5 protect animals and human beings from the consequences of septic shock". Cohen *et al.* teach that "anti-J5 antibody was significantly associated with protection from GvHD" (see page 8, right column).

N) Yoshioka *et al.* (*Res. Commun. Chem. Pathol. Pharmacol.* 80: 367-370, June 1993, abstract) teach that both an intraperitoneal injection and feeding of Rc mutant *E. coli* J5 LPS prevented endotoxin shock in suckling rats (see abstract).

O) Bhattacharjee *et al.* (*Clin. Res.* 41: p. 247A, 1993, already of record) reported the protection conferred in neutropenic rats against gram-negative bacteremia due to *Pseudomonas aeruginosa* by affinity-purified bacterin-induced J5LPS-specific IgG (see entire abstract). Note that two of the authors of this disclosure are listed as inventors of the instant application.

P) Cross *et al.* (*J. Endotoxin Res.* 1: 57-69, March 1994, already of record) reported optimal protection against gram-negative bacteremia conferred by an *E. coli* J5 bacterin-induced IgG eluted from an affinity column containing *E. coli* J5 LPS "devoid of lipid A" (i.e., delipidated or detoxified) and minimal protection conferred by the affinity column 'pass-through' which is enriched in anti-lipid A antibodies (see page 64). Cross *et al.* further state:

> Thus, these studies show that immunoglobulin fractions from post-immunization sera can mediate protection, and this protection is significantly diminished by removing immunoglobulin to ... J5 LPS[82]" (see page 64). Experimental studies described above achieved highly significant protection in animal models with antibodies directed against .... J5 LPS[82] (see page 64).

Q) Fenwick *et al.* (*Infect. Immun.* 53: 298-304, 1987, already of record) reported no deaths in the J5 bacterin-immunized pigs compared 85% of death among the non-immunized pigs. It is taught that "antibodies against common subsurface components of Gram negative bacterial cell walls correlate with protection from an otherwise lethal challenge with *H. pleuropneumoniae*" (see abstract).

R) Fenwick *et al.* (*Am. J. Vet. Res.* 47: 1888-1891, 1987, already of record) teach the benefit of increased immunity to cross-reacting LPS core antigens of Gram negative bacteria induced by vaccination with the Rc mutant of *E. coli* O111:B4 (strain J5). Compared to the

control animals, pigs vaccinated with *E. coli* J5 had lowered mortality with *H. pleuropneumoniae* infection (see abstract).

S) Tyler *et al.* (*Am. J. Vet Res.* 55: 1256-1260, 1994, abstract) teach the protection against enteric septicemia in catfish by immunization with a killed *E. coli* J5 vaccine (see abstract).

T) Ziegler *et al.* (*Clin. Res.* 29: 576A, 1981, abstract) teach successful treatment of human Gram-negative bacteremia with antiserum against endotoxin core using antiserum from humans vaccinated with heat-killed cells of *E. coli* J5 the LPS which contains a core region "which is similar in all gram-negative bacteria responsible for gram-negative bacteremia in man". 56% of immunized patients with profound septic shock recovered from shock compared to 29% of the control subjects (P=0.015). Ziegler *et al.* concluded that "antiserum to J5 endotoxin core substantially lowers the death rate from gram-negative bacteremia and septic shock" (see entire abstract).

U) In 1991, Tyler *et al.* (*J. Dairy Sci.* 74: 1235-1242, 1991) taught the following (see page 1236):

> Crossreactive immunoprophylaxis targeting Gram-negative bacteria has been extensively reviewed (3, 30). Passive and active immunization of laboratory animals against antigens common to Gram-negative bacteria have since demonstrated value in the prevention and treatment of experimental disease caused by heterologous bacteria (3, 30). The first reports of such cross-protective immunity in livestock included protection against *Salmonella typhimurium* enteritis, endotoxemia in calves, and *Actinobacillus* (*Hemophilus*) *pleuropneumoniae* in swine (8, 15, 16).
>
> Successful immunization hinges upon satisfying three basic criteria: 1) the vaccine must contain antigens common to all potential pathogens; 2) the common antigenic structure must be immunogenic, capable of inducing an immune response; and 3) this immune response must protect the host (29). The requirements of immunogenicity and protective effects have already been demonstrated by previous studies (3, 16, 18, 30). (See page 1241).
>
> Structures present in the inner core and lipid A portions of the cell wall are promising candidates as crossreactive immunogens......Based on our results, immunization with homologous core antigens may have utility in the prevention of coliform mastitis. (See page 1241).

In sum, the above-cited references published from 1982 through 1995 effectively rebut Applicants' statement that "all previous attempts to immunize or otherwise protect individuals against LPS endotoxin-mediated pathology had been unsuccessful" and that nearly a quarter of a century after the first publications cited by the Office, "there still is no vaccine to prevent or alleviate the severity of sepsis". This also highlights what a skilled artisan would glean from the dominant view in the art regarding the cross-reactive and cross-protective role of J5 LPS and

establish how it would have been obvious to a skilled artisan to use the readily available and the most studied cross-reactive/cross-protective *E. coli* J5 LPS to modify Zollinger's vaccine composition to produce the vaccine and the method of the instant invention. To characterize as the dominant view, the negative comments made by Greisman *et al.* in a review article that fails to list several important positive cross-reactive and/or cross-protective *E. coli* J5 studies, would be to mischaracterize the art. The review article of Greisman *et al.* is of little probative value.

10) Applicants contend that Ziegler herself was unable to correlate protection with J5 antibody titer, and that "many" others of skill in the art have commented on this point. Applicants contend that Myers' conclusion that Ziegler showed immunity against the core region is "faulty".

A thorough review of the prior art shows that Ziegler was able to correlate protection with J5-specific antibody. For instance, Ziegler *et al.* (*In*: *Seminars in Infectious Diseases*, Georg Thieme Verlag, New York, 1982, pp. 366-369) was able to show, via adsorption studies, that the protective antibody in the antiserum was directed specifically against the LPS core. Ziegler *et al.* also confirmed this "by showing that **fully cross-protective antiserum** could be prepared by **immunization** with **protein-free purified J5 LPS**" [Emphasis added] (see page 368). Ziegler *et al.* also teach:

> When compared to non-immune rabbit serum, J5 rabbit antiserum administered intravenously after the onset of *E. coli, Klebsiella or Pseudomonas* bacteremia strikingly enhanced survival rates even without antibiotics or other supportive measures. In experiments with survival rates less than 10% in animals given nonimmune serum, survival rose to 40 to 70 percent in those treated with J5 antiserum.[13,14] Antiserum to J5's parent *E. coli* O:111, whose core is concealed by side chains, was completely ineffective. (see page 368).

See also the teachings of Marks *et al.* (1982) above which also have shown the same by adsorption experiments.

Therefore, contrary to Applicants' arguments, Ziegler was able to correlate protection specifically with J5 antibody titer, and Myers' conclusion about Ziegler's demonstration of immunity against the core region does not appear to be "faulty".

Secondly, the Ziegler's studies, including the cited study, appear to be the most cited in the art. Many skilled in the art have commented positively on Ziegler's work. Two examples of positive comments about Ziegler's work are provided below:

a) McCabe *et al.* (*Progr. Clin. Biol. Res.* 47: 107-117, 1980, already of record) teach

(see page 110):

> Conformation of the protective effects of immunization with shared cross-reactive antigens of Gram-negative bacilli has been provided by Ziegler *et al.* These investigators demonstrated that both active and passive immunization with the J5 mutant of *E. coli* provided significant protection to granulocytopenic rabbits given lethal challenges of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* (Ziegler *et al.*, 1973; Ziegler *et al.*, 1974).

b) Tyler *et al.* (*Am. J Vet. Res.* 49: 1950-1954, 1988) teach (see page 1953):

> Active and passive immunization against gram-negative cell wall core antigens have demonstrated value in the treatment and prevention of gram-negative septicemia, bacteremia, and shock syndromes.[2,3,27-32]

Note that references 30, 31 and 32 are of Ziegler *et al.*

Interestingly, one of the recent publications, Bhattacharjee *et al.* (*Infect. Dis. Clin. North Amer.* 13: 355-369, June 1999) state (see page 361):

> In an attempt to determine whether antibodies to J5 LPS could have contributed to the protection observed in the Ziegler study, Bhattacharjee *et al.* have shown that antibodies produced in rabbits in response to immunization with a killed whole-cell *E. coli* J5 vaccine, when positively infused at onset of fever, protected neutropenic rats against lethal challenge with *P. aeruginosa* ....10. *E. coli* J5 LPS-specific IgG prepared from such sera protected 80% of neutropenic rats in a dose-dependent manner (Fig. 1).......These studies suggest **that IgG antibody elicited by a vaccine similar to the one used by Ziegler and colleagues could protect against heterologous sepsis when given as therapy.**

11) Applicants contend that the Lugowski's (1996) J5 antiserum did not show binding to *Klebsiella* and that Di Padova's (1993) LPS core-specific monoclonal antibody, "had no activity against *Klebsiella* or *Pseudomonas*" (see page 10 of the Applicants' response filed 04/18/2000). Applicants assert that Applicants' "J5 LPS/OMP vaccine does bind to *Klebsiella*" and "*Ps. aeruginosa*".

A close review of both the publications reveals that the J5 antiserum of Lugowski (1996) and the monoclonal antibody to DiPadova (1993) did show detectable binding to the LPS, or to the cells of *Klebsiella* sp. In fact, in a recent publication, Bhattacharjee *et al.* (*Infect. Dis. Clin. North Amer.* 13: 355-369, June 1999) acknowledge that DiPadova's WN1 222-5 monoclonal antibody does bind to some *Klebsiella* isolates (see page 361, first full paragraph). Note that Applicants' vaccine-induced antibodies were also tested for binding with some strains or isolates of *Klebsiella*.

It is unclear what Applicants mean by "J5 LPS/OMP vaccine" (as opposed to J5 LPS/OMP vaccine-induced antibody) binding to "*Klebsiella*". In any case, a review of the voluminous prior art on LPS reveals that there are sufficient numbers of publications, undisclosed to the Office by Applicants, which clearly demonstrate that J5-induced antiserum or antibody cross-reacts strongly with whole cells, or even with the purified LPS of multiple Gram negative bacteria, including *Klebsiella* and *Ps. aeruginosa*. For example:

- Tyler *et al.* (*J. Dairy Sci.* 75: 1821-1825, July 1992) teach highest cross-reactivity of an antibody reagent that recognizes homologous Gram negative core antigens (J5 antiserum affinity purified against endotoxin derived from an Ra mutant, *S. typhimurium*) with a chemically purified endotoxin of *Klebsiella pneumoniae*. The antibody reagent also cross-reacts with the chemically purified LPS of other heterologous Gram negative bacteria, such as *S. typhimurium*, *S. typhi*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *E. coli* O111:B4, O55:B5 and O26:B6 (see entire document, especially Figure 1, abstract, Results and Discussion). Tyler *et al.* state that the measurement of antibody reactivity against chemically purified LPS removes variables associated with capsular and somatic antigen masking of underlying homologous antigenic structures (see page 1822, left column, the last full paragraph).

- Tyler *et al.* (*J. Immunol. Methods* 129: 221-226, 1990) teach the cross-reactivity of an affinity purified immunoglobulin obtained from antisera raised in cattle to a killed *E. coli* J5 vaccine. The antiserum cross-reacted with the cells and chemically purified LPS of several heterologous Gram negative bacteria including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *S. typhimurium*, *S. dublin*, smooth *E. coli*, *S. newport* and *Bordetella bronchisepticum* (see pages 225 and 222).

- Tyler *et al.* (*J. Dairy Sci.* 74: 1235-1242, 1991) teach that affinity-purified antibodies raised against *E. coli* J5 cross-react strongly with a broad spectrum of Gram-negative bacterial whole cells including *Pseudomonas aeruginosa*, *S. typhimurium*, *S. dublin*, smooth *E. coli*, *S. newport*, *Klebsiella pneumoniae* and *Bordetella bronchisepticum*. Tyler *et al.* conclude that the "results suggest immunization with rough mutant bacteria may have broad application in

the prevention of coliform mastitis" (see abstract and page 1239).

- Salles *et al.* (*J. Infect. Dis.* 159: 641-647, 1989, already of record) teach monoclonal antibodies derived from mice immunized with *E. coli* J5 LPS and LPS-associated proteins. Seven monoclonal antibodies cross-reacted with the LPS of *E. coli* O111, O55, O127 and O128. One of the monoclonal antibodies, B7B3, cross-reacted with the LPS of *Serratia marcescens* and *Klebsiella pneumoniae* (see abstract).

- Nelles *et al.* (*Infect. Immun.* 46: 677-681, 1984, already of record) teach one or more antibodies induced by and reactive with *E. coli* J5 LPS, which "exhibit extensive serological cross-reactivity with a variety of gram-negative bacteria" (see abstract) including *Klebsiella pneumoniae* and *Ps. aeruginosa* (see Table 6).

- Cryz *et al.* (*Eur. J. Clin. Microbiol.* 4: 180-185, 1985) show that active immunization with an lipopolysaccharide derived from *E. coli* J5 induced IgG antibodies and afforded substantial protection against a strain of a heterologous Gram negative bacterium such as *Pseudomonas aeruginosa* (see abstract; Results and Table 2).

- Tomita *et al.* (*J. Dairy Sci.* 78: 2745-2752, 1995, already of record) teach the isolation of serum IgG from cows immunized with an *E. coli* J5 detoxified LPS conjugate vaccine and show that it is highly cross-reactive with the LPS of *E. coli* J5, *E. coli* O111:B4, *Serratia marcescens*, *Klebsiella pneumoniae* and *Salmonella typhimurium* (see abstract).

Thus, contrary to the Applicants' assertion, a *E. coli* J5 LPS or a detoxified J5 LPS vaccine has been shown to contain cross-reactive epitope(s) which induce antibodies that are reactive with multiple heterologous Gram negative bacterial pathogens including *Klebsiella* and/or *Pseudomonas* and their LPSs.

12) Applicants contend that despite "the widespread belief that J5 LPS is not an effective immunogen, Applicants persisted and have succeeded in providing the key to unlock J5 LPS's latent immunogenicity" and that "applicants have succeeded where others have failed" (see page 11 of the Applicants' response filed 04/18/2000).

As discussed above in paragraphs 9-11, a review of the prior art indicates that J5 LPS, with or without formulation with a carrier protein or an adjuvant, or an immunostimulant protein, was shown to be immunogenic and elicited cross-reactive and/or cross-protective antibodies. The cited prior art also shows that several skilled practitioners in the art persisted since Ziegler's publication, despite negative comments about Ziegler's work by a few, and succeeded in unlocking the immunogenicity of J5 LPS, before the effective filing date of the instant invention. That a number of skilled practitioners persisted in the art since Ziegler's publication and continued to study the role of anti-LPS antibodies in sepsis is evident from Applicants' own remarks made in a recent publication. Bhattacharjee *et al.* (*Infect. Dis. Clin. North Amer.* 13: 355-369, June 1999) state (see page 360):

> .... a number of subsequent studies did correlate survival from gram-negative sepsis with antibody to various core glycolipid antigens. Patients with high levels of anti-J5 antibody were more likely to survive *P. aeruginosa* sepsis [49]. In other studies, mortality from sepsis was correlated with depletion of antibody to anti-core glycolipid (Re) or to a mixture of core LPS antigens. [25,29,46,54] Such observations from different centers over extended periods of time served as an impetus to the continued study of anti-LPS antibodies for their potential useful in the prevention or treatment of sepsis.

13) Applicants contend that Dr. Cross's Declaration of 12 January 1999 provides the proof that a vaccine of the present invention indeed is effective and improves the outcome following a subsequent challenge with heterologous bacteria. Applicants state that the data provided in the Declaration are in distinct contrast to results achieved by passive immunization with antibodies.

First, the instant claims are drawn to a vaccine composition for use in a method of active immunization. Instant claims are not directed to passive immunization. Secondly, the Office has considered Dr. Cross's Declaration previously. In view of the applied art and what is well known in the art, the data provided in the Declaration are not unexpected. Since J5 LPS alone has been shown in the art to be a good immunogen, J5, with or without detoxification, when combined with a known effective protein carrier and a potent adjuvant such as group B meningococcal OMP, would be expected to be a more effective immunogen. Since J5 LPS or detoxified J5 LPS have elicited antibodies that are cross-reactive/cross-protective against heterologous Gram negative bacteria, such a J5 LPS, combined with a known and potent bacterially derived immunostimulant, such as group B meningococcal OMP, would be expected to induce antibodies that produce the same cross-reactive/cross-protective biologic effects. Since Applicants state that

their J5 LPS/*N. meningitidis* OMP vaccine, on immunization, neither prevents systemic infection, nor initiation of sepsis, but significantly reduces the likelihood of a lethal outcome following infections with heterologous strains of bacteria, such observations do not constitute "unexpected results".

Remarks

14) For the afore-mentioned reasons, claims 1-3, 5-8 and 15-17 stand rejected.

15) The prior art made of record but not relied upon in any of the rejections is relevant to the instant invention.

- Pollack *et al.* (*J. Clin. Invest.* 72: 1874-1881, 1983) teach:

> In this study, we showed a striking association between high concentrations of circulating antibodies to a well-characterized preparation of *E. coli* J5 core glycolipid present at the onset of *Pseudomonas* septicemia and subsequent survival. Our finding that levels of circulating antibody reactive with J5 core glycolipid are effective prognostic marker in *Pseudomonas* sepsis provides an important serological link between the known antigenic cross-reactivity of endotoxin core structures (5-7) and the protection recently observed in patients administered antiserum to the rough *E. coli* J5 mutant strain (18). The present study thus identifies an important immunological marker, which correlates with protective immunity in a common in a common form of Gram negative septicemia (see page 1879).

- Young *et al.* (*J. Clin. Invest.* 56: 850-861, 1975) immunized rabbits with purified glycolipid of "Re" mutant of *Salmonella minnesota* 595. Canine and alpine antiserum against glycolipid passively protected mice against a heterologous challenge. Antibody against core glycolipid protected against the hemodynamic sequelae of bacillemia, augmented intravascular clearance of serum-sensitive organisms and abrogated the pyrogenic response to enteric bacilli (see page 850). Young *et al.* teach the feasibility of protecting high risk human patients by immunization with CGL or related antigens, for these antigens seem to be considerably more versatile immunogens (see page 860).

- Ding *et al.* (*J. Med. Microbiol.* 31: 95, 1990) teach the protective immunity induced in mice by a detoxified salmonella lipopolysaccahride (see abstract).

- Nixdorff *et al.* (*In: Microbial Infections.* (Ed) Friedman *et al.* Plenum Press, New York, 49-61, 1992,) teach the enhanced immunogenicity of an LPS on complexing with a Gram negative bacterial cell wall protein. The preparation enhances IgG antibody-producing cell responses strictly specific for the serotype LPS used for immunization (see page 49).

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16) Any inquiry concerning this communication or earlier communications from the Examiner should be directed to S. Devi whose telephone number is (703) 308-9347. The Examiner can normally be reached on Monday to Friday from 8.00 a.m. to 4.00 p.m. A message may be left on the Examiner's voice mail system.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, James Housel, can be reached on (703) 308-4027.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

James C. Housel 5/29/00
JAMES C. HOUSEL
SUPERVISORY PATENT EXAMINER